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Transgenic mice with progressive degeneration of noradrenergic neurons restricted to locus coeruleus present depressive-like behavior and motor disturbances

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Background: Parkinson's Disease (PD) is characterized by the progressive degeneration of dopaminergic (DA) neurons of the substantia nigra (SN), however, other neurotransmitter systems are also affected. Substantial loss of noradrenergic (NA) neurons of the locus coeruleus (LC) is observed in the brains of many PD patients and it is hypothesized that this process may precede the detrimental changes in SN, which may manifest in depression or anxiety - non-motor symptoms of PD. The interaction between NA and DA neurotransmitter systems is intensively studied in the context of PD, however, most studies utilize toxin-based models, which induce rapid death of DA or NA neurons, however, do not reflect the progressive nature of the disorder. Previously we proposed a transgenic mouse model of progressive degeneration of NA neurons induced by conditional deletion of the gene encoding TIF-IA in cells harbouring dopamine β-hydroxylase (TIF-IA DbhCre). Ablation of TIF-IA in DBH expressing cells does not result in DA cell death in SN at 3 months, however, increased markers of inflammation and gliosis in SN were observed [1]. Due to the short lifespan of these animals caused by targeting peripheral tissues, further observations were not possible. Therefore, we created a new model combining CRISPR/Cas9 and Cre/loxP system to restrict the genetic modification only to LC.

Aim: To evaluate a transgenic mouse model of progressive noradrenergic degeneration restricted to LC, generated by the lentiviral vector (LVV) delivering CRISPR/Cas9 to DBH expressing cells, as a possible tool to study presymptomatic PD.

Methods: Anesthetised mice were injected with LVV in the LC region (A/P: -5.35; M/L: -0.90; D/V: -3.75). The efficacy of mutation was confirmed by 1) immunostaining of tyrosine hydroxylase (TH) in the region of LC, 2) HPLC measurement of NA content in the striatum. Behavioural testing was performed at two time points: 4 and 6 months after stereotaxic delivery of lentiviral vectors. Following tests were performed: open field, light-dark box, tail suspension (TST), rotarod and multiple static rods. The statistical significance was confirmed using t-student test; $p<0.05$ was considered significant.

Results: Immunostaining on LC sections confirmed loss of 30-50% of NA cells 6 months after stereotaxic surgery. HPLC studies revealed striatal content of NA reduced by 40% ($p=0.02$) in male mutant mice. Behavioural studies showed no changes in locomotor activity and anxiety in mutants. However, after 6 months mutant mice manifested increased immobility time in TST (by 72% $p=0,02$ in males and by 20% $p=0.003$ in females). Surprisingly, mutant males but not females, presented shorter endurance time on the rotarod (by 32%, $p=0.007$) and longer transit time on the static rod with the smallest diameter (by 58%, $p<0.001$) in multiple static rods test already 4 months after viral transduction, suggesting motor coordination disturbances.

Conclusions: These results suggest that progressive loss of NA innervation induces depressive behaviour in animals and impacts motor coordination, but in males only. This corresponds with the observation that a higher incidence rate of PD is diagnosed among men than women. Further biochemical and electrophysiological studies are needed to explain this phenomenon.

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Conflict of interest

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Beta-Synuclein protein function in behavioral impairment of triple knockout mouse model

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Introduction: Synaptic neurotransmission by dopamine (DA) is utilised by many neural networks and plays role in the development of pathological dependencies and cognitive disorders, including Parkinson's disease and certain types of addiction, e.g., alcohol addiction [1, 2]. Previous studies have demonstrated an importance of alpha-synuclein as a modulator of various mechanisms implicated in chemical neurotransmission. Information about beta- and gamma-synucleins involvement in molecular processes taking place in presynaptic terminals is limited [3]. All three members of the synuclein family have a high homology at the amino acid sequence level, which allows functional compensation within the family. Our recent results provided evidence that beta-synuclein but not alpha- or gamma-synuclein improves DA uptake by synaptic vesicles in dorsal striatum [4]. To further our understanding of the role of beta-synuclein in behaviour paradigm we used synuclein-free mouse lines in a set of tests.

Methods: Animals. Mouse lines: single knockout of beta-synuclein (b-KO) - B6(Cg)-Sncbtm1Sud, double knockout of alpha- and gamma-synucleins, produced by crossing (ag-KO) - B6(Cg)-Sncatm1.2Vlb and B6(Cg)-Sncgtm1Vlb lines, and a triple knockout (abg-KO) with genetic inactivation of all three synuclein genes, obtained by sequential multi-step crossing of the above 3 lines. The C57Bl/6J (Charles River) mice line with unmodified genome (WT) was used as a control. Animals were kept in specific pathogen free zone with standard conditions: 12/12 h light-dark cycle, temperature 20-22°C and free access to water and food.

Behavioural tests. Ageing (13-months old) mice balance and coordination were assessed using the basic motor tests – "Inverted grid" (IG), "Grip strength" (GS), "Accelerated rotarod" and Noldus CatWalk XT gait analysis system. Cognitive function was studied in test paradigms: "Open field" (OF), "Novel object recognition" (NOR), "Y-maze" (YM), "Morris water maze" (MWM) and "Elevated plus maze" (EPM) using Noldus EthoVision XT11 Software. Depressive-like behavior were studied in "Forced swim test" (FST).

Data analysis. GraphPad Prism 8 software was used for statistical analysis of experimental data by Kruskal-Wallis or ordinary one-way ANOVA tests. Normality was confirmed by Anderson-Darling test.

Results: Ageing mice without functional alpha- and gamma-synucleins displayed weaker limb strength in GS tests. In all cognitive tests abg-KO showed hyperactive phenotype compared to WT ($p<0.0001$). Activity parameters in OF, YM and EPM showed the same pattern: ag-KO which have only one functional beta-synuclein protein have no difference to WT, but absence of beta-synuclein protein in b-KO changes activity phenotype closely to abg-KO line. Comparative analysis of mouse lines showed that the presence of synuclein family proteins does not affect short-term memory formation in NOR and YM. Absence of beta-synuclein in b-KO caused faster memory formation in MWM long-term memory paradigm, but abg-KO showed lower memorization rate compared to WT mice. However, b-KO mice show similar behaviour to WT, while the absence of alpha- and gamma-synuclein decreased activity of ag-KO and abg-KO mice in FST, which may indicate more severe depressive-like behavior, but they show declined anxiety-like behavior in EPM.

Conclusion: Absence of beta-synuclein protein could influence hyperactive phenotype and memory formation but does not affect depressive-like behavior.

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To transfer a pharmacological neurovascular uncoupling model from mice to rats

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The objective of the present study was to establish a pharmacologically induced neurovascular uncoupling method in rats, as a translationally valid animal model of human cognitive decline.
The role and importance of neurovascular coupling in brain activity and cognitive function has come to the forefront of dementia and aging research. Diminished functionality of the neurovascular unit was shown during aging and in various brain disorders. Pharmacologically induced neurovascular uncoupling with subsequent neurological and cognitive defects was described in mice [1], however, no similar procedure has been reported so far in rats. Transferring the method to rats would extend its applicability to the most preferred species of the animal learning field.
In this study, we used 28 male Hannover Wistar rats. Neurovascular uncoupling was induced by intraperitoneal administration of a pharmacological "cocktail" consisting of N-(methylsulfonyl)-2-(2-propynyloxy)-benzenehexanamide (MSPPOH, a specific inhibitor of epoxyeicosatrienoic acid-producing epoxidases, 5 mg/kg), L-NG-nitroarginine methyl ester (L-NAME, a nitric oxide synthase inhibitor, 10 mg/kg) and indomethacin (a nonselective inhibitor of cyclooxygenases, 1 mg/kg) dissolved in 45% hydroxy-propyl-β-cyclodextrin (HPBCD), and injected twice daily for 8 consecutive days. Control animals received 45% HPBCD.
Animals were tested in the Morris water maze on days 5-7 of the treatment period, and in a fear-conditioning assay on day 4 (acquistion) and 8 (retention). Blood pressure of the animals was monitored non-invasively via tail cuffs, on days 1, 2, 5 and 7. Neurovascular coupling was measured in the barrel cortex in a non-recovery operation on day 8. A cranial window was fromed above the target area and a laser Doppler probe was used to detect changes in the animals' cerebral blood flow, while the contralateral whisker pad was mechanically stimulated, at an approximately 2 Hz frequency. Brain and small intestine tissue samples were collected post mortem and processed for prostaglandin E2 level measurements.
When contrasted with the control group, the animals treated with the pharmacological "cocktail" showed no significant changes in their performance either in the Morris water maze or the fear conditioning paradigm. However, we observed an overall higher blood pressure in these rats. They also showed about 50 % less increase in cerebral blood flow while their whiskers were stimulated, compared to the control group. Intestinal bleeding and ulcers were found in some of the treated animals and ELISA assays of the tissue samples revealed significantly decreased levels of prostaglandin E2 both in the brain and small intestine.
Although we could evoke neurovascular uncoupling by the applied mixture of pharmacons, it also induced adverse side effects such as hypertension and intestinal alterations. Furthermore, no significant impairment in the animals' cognitive performance was observed in the study. Thus, further refinements are still required for the development of an applicable model, mostly with regard to finding the appropriate dosages and learning assays.

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Cognitive characterization of the scopolamine-induced dementia model in experienced rats

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Scopolamine is an anticholinergic compound widely used as a pharmacological model of cognitive impairment. The drug is typically applied in single dose and in naïve or freshly taught animals. In a recent study we examined subchronic scopolamine treatment in a population of experienced aged animals with simultaneously testing the effect of the drug on three cognitive functions. We observed that the impairing effect of the compound was reversible within 9 days after cessation of treatment. The objective of the current study was to investigate the effects of repeated scopolamine treatment on several cognitive functions in young, experienced Long-Evans rats. We also tested to what extent donepezil could ameliorate the induced impairment.
Thirty-five male 8.5 months old Long-Evans rats were used in the study. The animals had been trained for/in the following tasks: 5-choice serial reaction time task (5CSRTT, measuring attention), Morris water maze (MWM, a spatial learning paradigm), pot jumping test (PJ, a motor learning task) [2], pairwise discrimination (PWD, an assay of visual learning) and a cooperation task (for testing social learning). All the rats routinely and regularly performed these task before the start of the study. After baseline measurements rats were randomly assigned into three treatment groups: saline (n=11), scopolamine (0.3 mg/kg ip., n=12), and scopolamine + donepezil (3 mg/kg ip., n=12). The saline and scopolamine groups received their appropriate treatment for 20 days, while the scopolamine + donepezil group was injected scopolamine for 10 days then scopolamine and donepezil during the following 10 days. The treatment period was followed by an 11-day wash-out phase. Cognitive performance of the rats was tested in the above described assays on 6 occasions: twice during each phase of the study (scopolamine only, scopolamine + donepezil, wash-out). Drug injections were carried out 30 min before the learning tests. Statistical significance ($p<0.05$) was determined by repeated measures ANOVA.
Scopolamine treatment caused differential effects on the studied cognitive domains. The compound did not exert significant effects in the MWM and PWD. In the 5CSRTT, control animals gave significantly more correct answers in the task and produced significantly less omissions compared to treated animals. During the cooperation tests, the scopolamine-treated group yielded significantly lower number of successful trials than the control group. However, these impairments gradually decreased during the treatment period. In PJ, the control group could jumped significantly longer distances compared to the scopolamine-treated groups, and in this paradigm – in contrast to the former two tasks – the magnitude of the scopolamine-effect increased by repeated treatments. Donepezil treatment did not ameliorate the learning performance deficit in any of the tests. All groups showed similar performance to their baseline levels already two days after discontinuation of the treatment.
Based on our results, scopolamine could not induce lasting changes in the functioning of cognitive neural networks, therefore it may not be an appropriate model for testing potential antidementia drugs, especially in young animals.

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